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Animal Model Selection for Inhalational HCN Exposure

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14. ABSTRACT

Cyanide (CN) is ubiquitous in many living organisms, commonly used in manufacturing processes (dyes, pigments, chelating agents, various nitriles, monomers, resins, fibers, case hardening, electroplating, extraction of precious metals, and fumigation), observed in accidental poisonings from natural or manmade products (smoke inhalation, cyanogens in apple seeds, peach pits, cherry pits, etc.) and as a possible terror threat agent. A higher priority has been placed on CN treatment and antidote evaluations in recent years, possibly as a result of CN being associated with fires, suicide, homicide, judicial execution, assassinations, chemical warfare operations, and as a terrorist threat. The purpose of this review is to identify the similarities and differences between human and animal species exposed orally to cyanide and provide documentation and justification for species model selection under the FDA Animal Rule. Review of CN absorption, metabolism, toxicokinetics, anatomic and physiologic assessment in various small and large animal species was conducted with focus placed on the advantages of each in CN research being provided in this review.

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Animal Model Selection for Inhalational HCN Exposure

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Abstract

Cyanide (CN) is ubiquitous in many living organisms, commonly used in manufacturing processes (dyes, pigments, chelating agents, various nitriles, monomers, resins, fibers, case hardening, electroplating, extraction of precious metals, and fumigation), observed in accidental poisonings from natural or manmade products (smoke inhalation, cyanogens in apple seeds, peach pits, cherry pits, etc.) and as a possible terror threat agent. A higher priority has been placed on CN treatment and antidote evaluations in recent years, possibly as a result of CN being associated with fires, suicide, homicide, judicial execution, assassinations, chemical warfare operations, and as a terrorist threat. The purpose of this review is to identify the similarities and differences between human and animal species exposed to inhalation cyanide and provide documentation and justification for species model selection under the FDA Animal Rule. Review of CN absorption, metabolism, toxicokinetics, anatomic and physiologic assessment in various small and large animal species was conducted with focus placed on the advantages of each in CN research being provided in this review.

Introduction

This document is intended to supplement the National Institutes of Health (NIH)-National Institute of Neurological Diseases Counter Measure Program for Cyanide (CN) with a paper focusing on the inhalational CN in the form hydrogen cyanide (HCN) and additional inhaled CN forms. Information specific to inhalational CN exposure, applicable animal data, and information supporting various species used in inhalation studies is presented.

CN is ubiquitous in many living organisms and is the toxic moiety. CN can be released in the form of hydrogen cyanide (HCN) during ingestion and metabolism of plant materials found in nature, such as peach pits, apple seeds, sorghum, corn stubble, etc. (Ballantyne et al, 2008; Casarett and Doull's Toxicology, 1986; EPA, 2010. CN^- is also released from HCN which is a component of a range of combustion processes of nitrogen containing materials (Lafferty, K.A. 2009; Alarie, Y., 2002). CN containing products are widely used in manufacturing processes, such as dyes, pigments, chelating agents, various nitrile monomers, resins, and fibers, case hardening, electroplating, extraction of precious metals,

and fumigation (Homan, E., 1987). Sources of toxicity and pathology information from CN include accidental poisonings from natural or manmade products, suicide, homicide, judicial execution, assassinations, and chemical warfare operations (WHO, 2004; Gee, D.J., 1987). CN was the first generation systemic warfare agent used during World War I and was used by Iran against Iraq (Baskin et al., 1997; Chemical Warfare Agents Toxicology and Treatment, 1996). Several public incidents have occurred that are indicative of intended CN use as a weapon; 10 tons of sodium cyanide were stolen in 2002, indictment of an individual for storing CN salts in a subway system, Homeland Security alert about potential use by al-Qaeda, and cyanide poisoning of a prominent Hong Kong businessman in 2012 (Keim, M.E., 2006; Newman, M., 2012). Analysis of mainstream smoke from one filter cigarette indicated 100 µg of HCN was released and the amount in non-filter cigarettes was 5 times that amount (WHO 2004, Pritchard, 2007). CN toxicity as a component of smoke inhalation or of fire-related morbidity and mortality was reported to cause 169 of 285 (59%) fire-related deaths (Grabowska et al, 2012). Deaths from CN toxicity related to smoke inhalation was estimated to cause 5,000-10,000 deaths annually in the US with mortality typically ranging from 24-31% (Erdman, A.R., 2007; Shepherd et al, 2007).

CN is one of many combustion by-products that can be divided into physical products and chemical toxicants. The physical products are mainly those of particulates and heat while the chemical toxicants include irritants (chlorine, etc.), simple asphyxiants such as carbon dioxide, and cellular asphyxiants, such as carbon monoxide, hydrogen cyanide, or hydrogen sulfide (Erdman, 2007; Shepherd et al, 2007).

There is a lack of controlled efficacy studies assessing the efficacy of approved CN therapies against smoke inhalation either in animals or humans, since most are antidotal in nature or reports of treatment of CN by other routes of exposure. The role that CN plays in smoke inhalation toxicity is unclear at best since the makeup of smoke varies with the combustion products. Thus, assessment and therapy of a burn/smoke inhalation patient is difficult and the therapeutic data available from treated victims is difficult to interpret in regards to degree of CN involvement in the morbidity and mortality (Erdman, 2007; Grabowska et al., 2012).

CN exposure can be rapidly fatal (within seconds to minutes), and is dose, time and exposure route dependent with inhalation being the most rapid, followed by intravenous infusion, oral then dermal

(cutaneous absorption) (Ballantyne et al, 2008; Ballantyne, B., 1983a). Systemic effects of CN intoxication are observed in organ systems most sensitive to low oxygen levels: the central nervous system, the cardiovascular system, and the pulmonary system (Salkowski et al., 1994; Yamamoto et al., 1982; Beasley et al., 1988); The CNS is the most sensitive target organ of CN poisoning with cardiovascular effects requiring higher CN doses than those necessary for CNS effects Yamamoto et al., 1982). Survivors may have long term neurological effects. Following acute inhalation exposure in humans and animals, cyanide is found in the lung, heart, blood, kidneys, and brain (Ballantyne, 1983, as cited in ATSDR, 2006).

Characteristics

Pure anhydrous HCN is a colorless liquid. HCN has a characteristic almond or bitter almond odor which can be detected at 2-5 ppm, however, the sense of smell fatigues easily. It has been reported that approximately 17% of the population cannot detect this odor due to genetic reasons, therefore, odor is not considered a reliable indicator of exposure (Marrs et al, 1987; Raza et al., 1994). Up to 40% of the population that cannot detect the odor has also been reported (Van Heijst, 1988). The most toxic form of CN is free CN, which includes the CN^- and HCN, either in gaseous or aqueous states. In solution at physiological conditions, the majority of HCN is largely undissociated resulting in rapid absorption through the high lipid content of the alveolar-capillary epithelium expedited by a large concentration gradient (alveolus to alveolar capillary) (Ballantyne et al., 2008; Medical Aspects of Chemical Warfare, 2008). HCN in the gaseous state has a vapor density of 0.948 compared to that of air with a vapor density of 1 (Raza et al., 1994). It has been noted that because of HCN's high vapor pressure and low density, a high concentration of HCN in open spaces is difficult to maintain. HCN's small size and moderate lipid solubility result in rapid absorption across mucous membranes. Table 1 presents limited physiochemical properties of HCN (Raza et al., 1994).

Mechanisms of Action

The mechanism of CN toxicity and cause of acute death are essentially the same regardless of exposure route. The same is true for acute and chronic/long term effects of CN intoxication; absorption, distribution, metabolism and elimination; in biological systems and end organ involvement, regardless of

exposure route (Chishiro, 2000; Pritchard, 2007). Any variation observed between routes of exposure is generally related to time of onset of observed signs with minor variation in signs observed and the progression of toxicity; all of which are strongly influenced by dose.

Table 1. Physio-chemical Properties of HCN

Physio-chemical Property	Description
Molecular Weight	27
Boiling Point	26.5 °C
Freezing Point	-13.4 °C
Vapor Pressure	807 mm Hg
Vapor Density	0.948
Specific Gravity	0.6969 (18 °C)
Volatility at 20°C	873,000 mg/m^3
Critical Temperature	138.5 °C
Critical Pressure	53.5 atm
Heat of Vaporization	210.7 cal/g
Solubility: Aqueous Organic Solvents	Dissolves to produce weak acid solution. Alcohol, ether, glycerol, chloroform, benzene.

HCN is readily absorbed through the lung due to its low molecular weight, moderate lipid solubility, poor ionization and rapid diffusion rate. CN via various routes of exposure including inhalation, intravenous, ingestion, or dermal causes cytotoxic- (intracellular), histotoxic- (pathological term) hypoxia Decreased utilization of oxygen leads to an increase in venous oxygen levels. Because the brain, heart, and other oxygen-sensitive tissue are impacted by CN, oxygen cannot be utilized in those tissues and results in cellular hypoxia. In addition, a decreased utilization of pyruvate by the mitochondria leads to anaerobic metabolism (decrease in ATP production, increase in ADP) and an increase in lactic acid production causing metabolic acidosis (Ballantyne et al., 2005; Wilson, 1983; Baud et al., 2002). Consequences of severe metabolic acidosis caused by lactic acidosis leads to CNS disturbances of perception and consciousness. The homeostatic mechanism to buffer lactic acidosis results in a progressive decrease in plasma bicarbonate. In severe CN poisoning, the effects are more complex with autonomic shock resulting from the release of biogenic amines and possibly CN inhibition of nitric oxide synthetase, also a heme-based molecule (nitric oxide plays a pivotal role in vascular tone and as a neurotransmitter) (NIOSH, Accessed 2013; Ballantyne, 1987a; Pritchard, 2007; Ballantyne et al., 2008]. Additionally, CN affects

calcium channels of the heart, resulting in increased intracellular calcium, hyperexcitability of cardiac muscle resulting in cardiac arrhythmia, vasoconstriction of the pulmonary arteriolar and coronary vessels, and a decrease in cardiac output. CN can also inhibit carbonic anhydrase, thus affecting neuronal transmission (Wilson, 1983; Pritchard, 2007). Other direct or secondary effects associated with CN are reacting with the ferric and carbonyl group of enzymes (e.g. catalase, peroxidase, phosphatase, tyrosinase, ascorbic acid oxidase, xanthine oxidase, hemoglobin), sulfhydryl compounds (e.g. cystine, mercaptopyruvate, glutathione), and proteins (e.g. iron containing proteins, carbonic anhydrase, succinic dehydrogenase, hemoglobin). Once bound, CN inhibits the reactions that these enzymes or proteins catalyze leading to blocking product formation. CN not only increases intracellular calcium, but generates reactive oxygen species, enhances N-methyl-D-aspartate (NMDA) receptor function, interacts with cystine to produce 2-ICA and 2-ACA (associated with memory loss, convulsions), elicits dopaminergic toxicity, produces lactic acidosis (by-product of anaerobic metabolism), mitochondrial ADP ribosylation, and hyperammonemia (NIOSH, Accessed 2013; Ballantyne, 1987a; Pritchard, 2007; Ballantyne et al., 2008). CN inhibition may be due to affinity to Schiff base intermediates (e.g. ribulose diphosphate carboxylase) and 2-keto-4-hydroxy glutarate aldolase involving formation of a cyanohydrins intermediate (Marrs et al, 1987; Raza et al., 1994). CN alters carbohydrate metabolism resulting in increased glycogenolysis, shunting of glucose to the pentose phosphate pathway by decreasing the rate of glycolysis, and inhibition of the tricarboxylic acid cycle with marked metabolic acidosis (Ballantyne, 1987a). At high atmospheric concentrations of HCN, there is a marked concentration gradient across the alveolar capillaries resulting in rapid absorption of HCN in the lung and rapid death without first-pass liver detoxification. Since HCN rapidly diffuses into circulation and is minimally detoxified by either the liver or other detoxification pathways due to the marked concentration gradient, the sensitive tissues and target organs attain toxic CN concentrations rapidly (Ballantyne et al., 2008; Ballantyne, 1987a; Pritchard, 2007).

Clinical Presentation

Exposure to high concentrations of HCN can result in death within seconds to minutes. Lower concentrations or prolonged low exposures show a progression of signs and symptoms that are related to

progressive intracellular hypoxia. The hallmark presentation is hypoxia and acidosis (Gracia et al., 2004). In humans, the inhalation of CN as HCN has a lethality estimated at 2500-5000 mg-min/m^3. CN is detoxified at a rate of about 17 µg/kg-min. As a result, the lethal concentration per a given time in 50 percent of the population (LCt_{50}) is greater for a long exposure (e.g., 60 min) than for a short exposure (e.g., 2 min) (Ballantyne, 1987a; Pritchard, 2007). The onset and progression of signs and symptoms after inhalation of a lower concentration of vapor are slower and similar to the ingestion of CN. The first effects may not occur until several minutes after exposure, and the time course of these effects depends on the amount absorbed, the rate of absorption, and first-pass liver detoxification effects. Common signs and symptoms presented after inhalation of HCN include an initial transient hyperpnea followed by feelings of anxiety or apprehension, agitation or restlessness, puritis, eye irritation, facial flushing, vertigo, headache, weakness, nausea emesis, chest tightness, confusion, and muscular trembling. A progression of signs leads to dyspnea, cyanosis, hypotension, bradycardia, syncope, decreases in rate and depth of respiration, opisthotonus, apnea, cardiac dysrhythmias, and death (Lam et al., 2000; Chishiro, 2000; Campbell, 2000; Medical Management of Chemical Casualties Handbook, 2007; Bhattacharya et al., 2009).

At higher concentrations, death may occur in seconds to minutes and signs may be no more than collapse and/or coma depending on concentration and time. While cardiac irregularities are often noted, cardiac function invariably outlasts respiratory function. Death is typically due to respiratory arrest of central origin (Salkowski et al, 1994). The toxicity of HCN in humans is dependent on the nature of the exposure. Due to the variability of dose-response effects between individuals, the toxicity of a substance is typically expressed as the concentration or dose that is lethal to 50% of the exposed population (LC_{50} or $LD_{50,}$ respectively). The LC_{50} for gaseous HCN is 100-300 parts per million for humans (Ballantyne, 1987a). Inhalation of CN in this range generally results in death within 60 minutes, with death coming more quickly as the concentration increases. Inhalation of 2,000 parts per million (ppm) HCN can result in death within one minute (Pritchard, 2007).

HCN inhalation produces incapacitation and an inability to escape from the environment (Ballantyne, 1987, Purser et al., 1984). A study conducted in rats indicated incapacitation concentrations

were approximately 65% of the lethal concentrations (Levin et al., 1987). Table 4 presents the inhalation toxicity of HCN in various species.

Table 4. Representative Lethality Values for Hydrogen Cyanide

Species	LC_{50} mg/m^3	Duration of Exposure (min)	Reference
Human	150	30 min	WHO, 2004
	200	10 min	
	300	Immediate	
Mouse	176	30 min	Matijak-Schaper and Alarie, 1982; Ballantyne, 1987a
	363	5 min	MAK 2003[5]
	1129	1 min	Ballantyne, 1984b; Ballantyne, 1987a
Guinea Pig	400	NA	Pritchard, 2007; WHO, 2004
Rabbit	208	35 min	Ballantyne, 1984b; Ballantyne, 1987a
	409	5 min	
	2432	45 sec	
Canine	100	NA	Pritchard, 2007; WHO, 2004
Swine	3404	1	McNamara, 1976
	1466	3	
	607	10	
	688	90	
NHP	96-217[1] (87-196 ppm)	30	Purser et al, 1984; RAIS, Accessed 2013
	110-172[1], (100-156 ppm)	8-19	Pritchard, 2007; Purser et al, 1984
	110-172[1], (100-156 ppm)	8-19	Purser et al, 1984; Ballantyne, 1987a

[1]Values in mg/m^3 were approximate calculations from ppm where mg/m^3 = ppm x gram molecular weight/24.45(molar volume of air at standard temperature and pressure).

Health Effects from CN Exposure

Cardiovascular responses to CN are complex and include precordial pain and EKG abnormalities. Studies of isolated heart preparations and intact animal models show that principal cardiac insult is slowing of rate and loss of contractile force. Several reflex mechanisms, including catecholamine release and central

vasomotor activity, may modulate myocardial performance and vascular response in patients with cyanide poisoning. In laboratory investigations, a brief period of increased inotropy caused by reflex compensatory mechanisms occurs before myocardial depression. Clinically, an initial period of bradycardia and hypertension may occur, followed by hypotension with reflex tachycardia, but the terminal event is consistently bradycardia and hypotension. Ventricular dysrhythmias do not appear to be an important factor. Severe poisoning cases result in the patient developing shock, cardiac arrest, hypoxic myocardial ischemia, myocardial dysfunction, conduction disorders, and arrhythmias (atrial fibrillation, and extrasystole). Severe metabolic acidosis occurs in association with an increase anion gap.

Both cardiogenic pulmonary edema and acute lung injury are found at necropsy. In CN poisoning, acute lung injury may be neurogenic in origin or result from membrane leak from direct pneumocyte toxicity. Inhalation of HCN may be associated with mild corrosive injury to the respiratory tract mucosa. Survivors of serious acute poisoning may develop delayed neurologic sequelae. Parkinsonian symptoms, including dystonia, dysarthria, rigidity, and bradykinesia, are most common. Symptoms typically develop over weeks to months, but subtle findings can be present within a few days. Cranial computerized tomography and magnetic resonance imaging consistently reveal basal ganglia (globus pallidus, putamen, and hippocampus) damage, with radiologic changes appearing several weeks after onset of symptoms. Extra pyramidal manifestations may progress or resolve. Response to pharmacotherapy with anti-parkinsonian agents is generally disappointing. Whether delayed manifestations result from direct cellular injury or hypoxia is unclear (Ballantyne et al., 2008; Pritchard, 2007; Baskin et al., 1997; Van Heijst, 1988; Bhattacharya et al., 2009; Beasley et al, 1988; Gracia et al., 2004).

Some of the chronic effects reported are central nervous system (CNS) effects, thyroid enlargement and hematological disorders in humans (Blanc et al, 1985; Chandra et al, 1980; El Ghawabi et al, 1975). Chronic exposures to workers at 15 ppm HCN with unknown duration reported the following range of effects: fatigue, dizziness, headache, disturbed sleep, and tinnitus. Neurological effects have been reported to persist on cessation of chronic exposure (Pritchard, 2007 and ATSDR, 2004). However, survivors of moderate to high level intoxication have reported long term health effects especially from ingestion of CN (Ballantyne et al., 2008; Ballantyne, 1987a). While multiple systems (CNS, respiratory, cardiovascular,

hematological, and thyroid) may be affected, the brain is selectively sensitive given its high oxygen consumption and low rhodanese content, an enzyme involved in CN detoxification (Ballantyne et al., 2008; Baskin et al, 1997; Aminlari et al., 2007). The cortical gray matter, hippocampus, corpora striata, and substantiation nigra are commonly affected. CN has also been shown to damage white matter (Levine, 1967). Such effects include a decrease in brain gamma-aminobutyric acid, encephalopathy, and demyelinating lesions as a result of CN or hypoxia. Ultrastructural changes in heart muscle and thyroid changes, such as frank goiter, have been documented (Van Heijst, 1988; Yamamoto et al., 1982).

Chronic exposure to cyanide is also associated with thyroid disorders. Thiocyanate is a competitive inhibitor of iodide entry into the thyroid, thereby causing formation of goiters and the development of hypothyroidism. HCN is not considered to have significant mutagenic potential and is not a carcinogen (Banea-Mayambu et al., 1997; Ballantyne et al., 2008; NIOSH, Accessed 2013; ECETOC, 2004; World Health Organization, 2013).

Disposition of Inhaled CN

Clinical presentation of CN intoxication is dependent on the accumulation of CN in body fluids and tissue target sites in sufficient quantity to adversely affect biochemical and target intracellular sites to overwhelm detoxification and elimination pathways enough to result in overt signs of CN intoxication. Thus, CN intoxication is dependent on a number of factors including the rate of absorption, the exposure dose, the distribution within the body, and the detoxification rate (Ballantyne, 1987a).

The rate of absorption is influenced by the chemical and physical nature of the exposure material. Because HCN has a low molecular weight and is non-ionized, it is absorbed at a greater rate than KCN, which has a higher molecular weight and is ionized. The exposure dose is related to the amount of CN the patient is exposed to, the exposure concentration, the exposure time and the number and frequency of exposures. The distribution of CN is affected by the route of exposure as there are differences in regional blood supply and differences in binding affinity of CN for different cellular structures and macromolecules. The rate of detoxification relative to the rate of CN absorption dramatically influences the rate of CN accumulation. The rate of toxicant supply to the tissues for detoxification and the availability of detoxifying

substrates (such as sulfur donors) greatly influence the endogenous detoxification process (Ballantyne, 1987a).

Lethality data from various exposure periods (generally within an hour) indicates a disproportionate relationship between exposure time needed to induce death in a selected proportion of the test population and the concentration needed to induce that degree of mortality. In general, for short exposure durations (several minutes), as concentration decreases, only small increases of time are needed to obtain moderate to large decreases in lethal concentration in 50 percent of the population (LC_{50}) (Ballantyne, 1987a). The total dose of HCN leading to death is disproportionately larger at low concentrations than at high concentrations, thus the time to death is disproportionately longer (WHO, 2004). The disproportionately greater detoxification of CN at lower levels of delivery is the cause of this effect (Ballantyne, 1987a).

Absorption

HCN is small in size and moderately lipid soluble, favoring rapid absorption across mucous membranes and alveolar epithelium uptake before rapid distribution throughout the body. High atmospheric HCN concentration exposures develop a marked concentration gradient across the alveolus-alveolar capillary epithelium resulting in rapid absorption of HCN. The rapidly absorbed HCN and the high concentration of HCN circulating in the blood stream is observed also in the oxygen sensitive target organs and tissues minimizing the liver's first pass detoxification effect and resulting in rapid CN intoxication with severe poisoning or death within seconds to minutes. Lower atmospheric HCN concentration exposures absorb into the systemic circulation at a slower rate allowing for a first pass effect in the liver and detoxification processes. This results in a slower rise in body burden of toxic levels of CN demonstrating a delay in toxicological onset of signs and symptoms and death (Ballantyne et al, 1987a).

Distribution

Distribution of the absorbed CN occurs rapidly throughout the body. CN enters the erythrocyte (RBC) where it is sequestered. This results in higher CN^- levels in the RBC than in the whole blood, sera or plasma in all species. Purser found that for the first 10 minutes of a 30 minute HCN exposure in

cynomolgus monkeys at 102 to 156 ppm, there was a good linear correlation (r=0.94) between HCN exposure concentration and blood cyanide. Allowing for inter-animal variation in respiratory minute volume, a plateau for whole blood CN is reached at around 10-15 minutes (Purser et al., 1984).

Ballantyne demonstrated in rabbits that tissue values of CN following acute HCN vapor exposure were lower than those corresponding tissues from animals administered lethal doses of HCN by alternate routes. Those tissues showing the highest level of CN and of consistent diagnostic concentrations of inhaled CN were blood, brain, heart, and lung. Liver levels of CN were low or undetectable (Ballantyne, 1987a; Ballantyne, 1984a; Ballantyne, 1984b; Ballantyne, 1983a). CN^- has been shown to readily cross the placenta so maternal exposure to high concentrations of CN^- may also be toxic to the fetus (Levin et al., 1987).

Metabolism and Elimination

The detoxification of CN in the body occurs through several pathways while the elimination of CN is the same regardless of the route of exposure. CN is primarily metabolized by the formation of the less acutely toxic compound thiocyanate (SCN), primarily by rhodanese, and some by 3-mercaptopyruvate sulfur transferase (MPST). Conversion to SCN occurs with up to 80% of a CN dose. Rhodanese is a mitochondrial enzyme that transfers a sulfur atom to CN from a sulfur-sulfur donor, such as glutathione, thiosulfate, or cystine. Another enzyme, mercaptopyruvate sulfurtransferase, which is found in both mitochondria and the cytosol, transfers sulfur from the organic thiols, mercaptopyruvate and thiocystine, to CN (Ballantyne et al., 2008; Ballantyne, 1987a; Pritchard, 2007). A minor pathway of metabolism involves the spontaneous reaction of CN with cystine forming 2-aminothiazoline-4-carboxylic acid. This pathway accounts for about 15% of CN metabolism in rats. A high level of rhodanese activity is found in liver and skeletal muscle. Given the higher mass of skeletal muscle, the amount of CN detoxified by skeletal muscle is about 2.6-fold more than liver (Handbook of Toxicology of Chemical Warfare Agents, 2006, 2009).

Animal and human data indicates that CN is primarily eliminated in the urine as SCN following either oral or inhalational exposure. The SCN formed is excreted by the kidneys and has a half-life of about 2.7 days in healthy humans (Ballantyne et al., 2008; Ballantyne, 1987a; Schulz et al., 1979; Schulz, 1984; Ansell et al., 1970).

Rhodanese, the primary source of CN metabolism, follows zero-order kinetics with the concentration of sulfur donor as the rate limiting step (Handbook of Toxicology of Chemical Warfare Agents, 2006, 2009). In this regard, the dog may not be an appropriate model for predicting human response due to the significantly lower level of rhodanese in dogs. In general, the dog is known to have low levels of hepatic and plasma rhodanese, the nonhuman primate intermediate levels and the rat high levels of activity (Drawbridge et al., 1987). Due to zero-order kinetics, some differences in rhodanese activity between species are not likely to translate to a difference in sensitivity to CN toxicity. Dahl reported that rhodanese was in high concentrations in rat nasal tissue and in particular the olfactory region. A seven-fold increase (per mg mitochondrial protein basis) over that of the liver was detected (Dahl, 1989). In addition, the nasal cavity of the dog demonstrated higher rhodanese activity than other sections of the respiratory system (Aminlari et al, 1994). Lewis found that on a per mg mitochondrial protein basis, the rhodanese in human nasal tissue exhibited both a lower affinity (higher Km) for CN and a lower maximum velocity (Vmax) for CN metabolism than did rhodanese from rat nasal tissue (Lewis et al., 1991).

The enzyme, 3-mercaptopyruvate sulfurtransferase (3-MPST; 3-mercaptopyruvate: cyanide sulfurtransferase), located in the cytoplasm, mitochondria, and blood, is involved in the detoxification of CN. The substrate 3-mercaptopyruvate (3MP) is hydrolyzed by 3-MPST releasing sulfane which binds CN forming the less toxic product, thiocyanate (SCN). Sulfanegen is a substrate for 3-MPST and acts as an additional, exogenous source of sulfane which can be used to expedite CN detoxification (Ballantyne et al., 2008; Ballantyne, 1987a; Pritchard, 2007; Baumeister et al., 1975; Westley et al., 1983). Figure 1 presents the 3-MPST activity in blood for multiple species (courtesy of Dr. Stephen Patterson). The rat, rabbit, and NHP have similar 3-MST activity to that reported in humans, and would be anticipated to respond in a similar manner to humans.

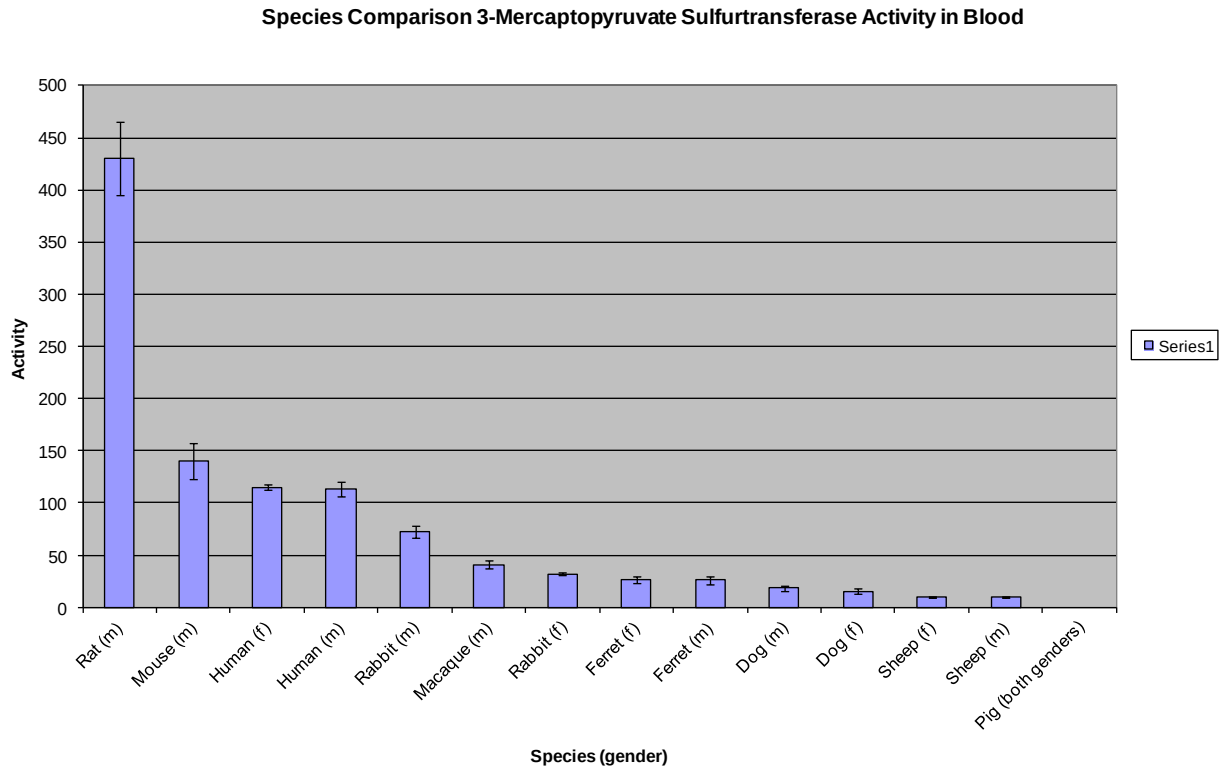

Figure 1. Graphic Representation of Species Blood Comparison of 3-MPST Activity[60]

Enzyme Units of 3-mercaptopyruvate sulfurtransferase are defined as µmoles of pyruvate generated per minute per 10^{10} RBCs at 37 ºC. (Courtesy of Dr. Stephen Patterson).

Animal Models for CN Research

Many animal species have been used in inhalation CN research studies in support of human clinical studies (Handbook of Laboratory Animal Science, 2005; Laboratory Animal Medicine, 2002, 1984). There are three main groups of animals with anatomically similar respiratory tracts: Group 1 consists of cattle, sheep and pig; Group 2 consists of dog, cat, monkey, rat, rabbit and guinea pig; and Group 3 consists of horse and man. The anatomy of the respiratory tract varies markedly between the species in the following features: 1) shape of upper and lower respiratory tract; 2) extent, shape and pattern of turbinate bones; 3) branching patterns of bronchi; 4) anatomy of terminal bronchioles, including collateral circulation; 5) lobation and lobulation; 6) thickness of the pleura; 7) completeness of the mediastinum; 8) relationship of pulmonary arteries to bronchial arteries and bronchioles; 9) presence of vascular shunts; 10) distribution of

mast cells; and 11) blood supply to the pleura (Merck Veterinary Manual, 1998). The understanding of physiological, anatomical, and biochemical differences between the respiratory and cardiopulmonary system of different animal species can lead to the selection of the correct animal model to mimic the bioavailability of compounds in the human (Holstege et al., 2006). The cell types that comprise the pulmonary system are described for all species under the nonhuman primate section. The percentage of these cell types in various regions of the lung varies with species and this information is provided in the section under each species.

Mice

Mice have been used in scientific research since the 1600s. Their development into a laboratory mouse as a research model began with genetic experiments in the 1900s. Other types of research uses include, but are not limited to behavioral research, genetic, cancer (spontaneous and genetically engineered), immunology, toxicology, pharmacology and bioassay, metabolism, developmental biology, embryology, diabetes, obesity, aging, convulsive disorders, ophthalmology, infectious disease (bacterial, fungal, parasitic, viral), monoclonal antibody production, and cardiovascular research (Laboratory Animal Medicine, 2002, 1984; Morse, 1981; The Mouse in Biomedical Research, 1982) . Normal ranges for physiological values vary with strain.

The respiratory system of a mouse is divided into three main areas: 1) The cranial respiratory tract consists of the nostrils, nasal cavities, and nasopharynx; 2) the intermediate section consists of the larynx, trachea, and bronchi; and 3) the caudal section of the respiratory tract consists of the lungs. The intermediate section has cartilaginous support. The lung consists of a left lung lobe that is a single lobe and the right lung lobe that consists of four lobes: the superior lobe, middle lobe, inferior lobe, and postcaval lobe (Laboratory Animal Medicine, 2002, 1984; Morse, 1981; The Mouse in Biomedical Research, 1982). The lung volume of mice is approximately 4 percent (lung volume/body weight) (Ménache et al, 1997; Harkema et al., 1987a, 1987b, 1987c). The tracheobronchial airway comprises 11 percent of the lung in the mouse with 13-17 generations (Bal et al., 1988).

Mice have a very high metabolic rate and, at rest, use about 3.5 mL O_2/gm/hr. To maintain such a high metabolic rate, the mouse has: a high alveolar PO_2, a rapid respiratory rate, a short air passage, a moderately high erythrocyte (RBC) concentration, high RBC hemoglobin and carbonic anhydrase concentrations, a high blood O_2 capacity, high capillary density, and a high blood sugar concentration (Handbook of Laboratory Animal Science, 2005; Laboratory Animal Medicine, 2002, 1984; Gomes et al., 2000).

Rats

Rats have been widely used in respiratory safety pharmacology studies resulting in a well-documented and characterized respiratory system and a plethora of scientific publications of drug effects on the respiratory system. The larger size of rats, compared to mice, allows additional sampling (blood, cerebrospinal fluid etc.) from the same animal. Like mice, the rat's normative physiologic data varies between sources and strains, or stocks.

Rats, similar to rabbits, are obligate nose breathers (Laboratory Animal Medicine, 2002, 1984). The rat's nasal cavity is involved with respiration, humidification of air, and filtering of particles. Approximately 50 percent of the nasal cavity is lined with olfactory epithelium, hence an acute sense of smell. The vomeronasal organ lies approximately 10 mm from the vestibule in the ventral vomer bone and is involved with olfaction also. The rat has a maxillary recess (sinus), located between the maxillary bone and the lateral lamina of the ethmoid bone. Within this recess is the commonly termed Steno's gland or lateral nasal gland, the largest of several nasal glands. This gland secretes a watery discharge at the rostral end of the nasal turbinate with the duct emptying into the vestibule (McLaughlin et al., 1961; Ménache et al., 1997). The secretion may act to regulate the viscosity of the mucous layer overlying the nasal epithelium and humidify the air (Handbook of Laboratory Animal Science, 2005).

The respiratory system in the rat is similar to the mouse. The left lung is a single lobe and the right lung consists of 4 lobes: the cranial, middle, accessory and caudal lobes. The diameter of the trachea is 1.6-7.7 mm for an average adult rat with a length of approximately 33 mm from first cartilage to bifurcation. The mean alveolar diameter has a mean of 70 µm and a range of 57-112 µm. The thickness of the air-blood

barrier is 1.5 µm. There are 2-5 branches per alveolar duct. The lung volume in the rat is approximately 3 percent (lung volume/body weight). The tracheobronchial airway comprises 5.7 percent of the lung in the rat with 13-17 generations (Laboratory Animal Medicine, 2002, 1984; Bal et al., 1988; Meyer et al., 1989; McLaughlin et al., 1961; Handbook of Laboratory Animal Science, 2005).

The precapillary anastomosis occurs in the hilar region, the same as in man. In the rat, the pulmonary vein has cardiac striated muscle fibers within its walls, which are continuous with those of the heart. The pulmonary artery is the thinnest and the pulmonary vein is the thickest due to the cardiac striated fibers, which are predominantly in the intrapulmonary branches. Infections can readily spread from the heart to the lungs by this arrangement. There is no adrenergic innervation to the bronchial musculature so bronchoconstriction is controlled by vagal tone (Laboratory Animal Medicine, 2002, 1984). Rats have high neuronal density, high serotonin activity, and low histamine activity in the lungs. Pulmonary vasculature responds to acetylcholine at doses of 0.2-0.5 µg (Shapiro, 2006; Sakuma et al., 1997).

At least ten morphologically distinct cell types have been identified in the pulmonary airways. The epithelial serous cells are thought to be unique to the rat. These cells secrete a substance whose viscosity is less than that of mucous cells. These cells are believed to be responsible for the low-viscosity pericilliary liquid layer found at all levels of the rat's respiratory tract (Handbook of Laboratory Animal Science, 2005; Ménache et al., 1997). The olfactory epithelium (sensory cells or receptor cells) provides the acute sense of smell noted in the rodent species, since it covers approximately 50 percent of the nasal cavity surface. The turnover rate of olfactory sensory cells for the rat is 28-30 days. The sustentacular cells are support cells for the olfactory sensory cells and are noted for metabolism (xenobiotic, etc.). Squamous epithelium, transitional epithelium, respiratory epithelium, vomeronasal organ, and immune tissues also comprise the cellular types of the pulmonary system (Gross et al, 1982).

The transitional respiratory epithelium in rodents is 1-2 layers thick (considered thin), pseudostratified, and composed of three cell types (basal, cuboidal, and columnar) (Monteiro-Riviere et al., 1984 and Harkema et al., 1987a, b, c). The luminal nonciliated cells of the transitional epithelium in rodents have abundant smooth endoplasmic reticulum (SER) and no secretory granules (Harkema et al., 1987a, b, c). Although minimal to no secretory granules are reported in rodent or monkeys, if exposed to high ambient

concentrations of irritating pollutants, a rapid increase in numerous mucous-secreting cells occurs (Monteiro-Riviere et al, 1984; Harkema et al., 1987a, b, c; Harkema et al., 1999). Respiratory epithelium (ciliated respiratory cells) lines approximately 46 percent of the rat nasal cavity (Gross et al., 1982). Respiratory epithelium of the rat is consistent with that of other mammals, being composed primarily of ciliated cells, mucous goblet cells, and basal cells (Harkema et al., 1987b). Brush cells are found in nasal respiratory epithelium of rats but are not present in monkeys. The vomeronasal organ (Jacobson's organ) in most mammals is located in the vomer bone in the ventral portion of the proximal nasal septum and is a paired tubular diverticulum, a chemosensory structure that contributes to the sense of smell in macrosmatic species, such as: rodents, dogs and rabbits. In the laboratory rodent, the lateral wall of this organ is lined with tall columnar, respiratory-like epithelium (nonchemosensory). The medial wall is lined with sensory neuroepithelium (chemosensory) morphologically similar to olfactory epithelium of the nasal cavity. Discrete larger focal sites of lymphoid tissue termed nasal associated lymphoid tissue (NALT) are located in the nasopharyngeal mucosa of humans, laboratory animals and primates (Handbook of Laboratory Animal Science, 2005; and Harkema et al, 1987a, b, c).

Tissue CO_2 exchange in the medullary respiratory center is believed to regulate the respiratory system of the rat with the carotid bodies playing a role. The carotid bodies respond to low blood oxygen tension (West et al., 1965).

At birth, the lung is immature and, as a consequence, is lacking in alveoli, alveolar ducts, and respiratory bronchioles. Air exchange is through smooth walled channels and sacculus until 4-7 days of age at which time remodeling occurs and at 10 days of age, the respiratory bronchioles are present (Yokoyama, 1983).

Guinea Pigs

Guinea pigs have been used as models of lung–function impairment and bronchial reactions such as airway hyper-responsiveness and reaction similar to human asthma (Terril et al., 1998; Brewer et al., 1997; Nagases et al., 1994; Martin, 1994; Cook et al, 1998). The caudodorsal part of the nasal cavity is lined with olfactory epithelium, resulting in a keen sense of smell. The larynx is typical of all mammals consisting of 5 cartilages, but there is no laryngeal ventricle. Despite a wide range of vocal sounds the

guinea pigs, vocal cords are small and poorly developed. The caudal tongue is continuous with the soft palate except for the palatal ostium (inter-pharyngeal ostium) located in the middle. This aperture forms the connection between the oropharynx and pharynx. The soft palate folds around this hole are called the velo pharyngeal recess. The lungs of the guinea pig are divided into seven lobes; the four right lobes are termed cranial, middle, caudal, and accessory lobes; and the three left lobes are termed cranial, middle and caudal. The prominent perivascular lymphoid nodules surrounding the branches of pulmonary arteries and veins in most guinea pigs tend to increase in size as the animal ages. These nodules can become large enough to be grossly visible at necropsy and function has not yet been determined (Laboratory Animal Medicine, 2002, 1984; The Biology and Medicine of Rabbits and Rodents, 1995). The bronchi are highly reactive (sensitive) to histamine resulting in spasms during immediate hypersensitivity reactions. Distinguishing anatomical characteristics to this effect have not been identified (Shapiro, 2006; Bauer et al., 2007; Nagase et al., 1994; The Biology and Medicine of Rabbits and Rodents, 1995).

Rabbits

Rabbits are obligate nose breathers. In the upper respiratory tract, rabbits have sensory pads just inside of the nostrils, making them very sensitive to touch. Twenty to twenty-five tactile vibrissae are located on either side of the upper lip. When the animal is fully relaxed the nostrils are still, otherwise there are 20 to 150 nostril twitches per minute. Rabbits have an acute sense of smell due to the nasal turbinates having the vomeronasal organ and olfactory epithelium. The rabbit glottis is small and covered frequently by the tongue. Intubation of rabbits is difficult due to the small glottis, long tongue, narrow oropharynx, and laryngospasm. In the lower respiratory tract, the thorax is small compared to the large abdomen. The rabbit lung consists of six lobes; both right and left lobes are divided into cranial, middle and caudal lung lobes with the left cranial lobe much smaller than the right due to the presence of the heart, and the right caudal lobe being subdivided into the lateral and medial portions. Rabbits have very thin pleura and unlike other domestic species, there are no septa dividing the lung into lobules. Thus pneumonia in rabbits is not as localized as in most species (Nowak, 1999; The Biology and Medicine of Rabbits and Rodents, 1995; Laboratory Animal Medicine, 1984).

Muscular contraction and relaxation of the diaphragm is responsible for respiration and not the intercostal muscles or musculature of the thoracic wall. Artificial respiration in rabbits is easily conducted by holding the animal and alternating the head and body between an up position and down position, 30-40 times a minute, to stimulate diaphragm movement. Flow volume of air to the left lung is greater than the right due to lower resistance of proximal airways per unit volume (Handbook of Laboratory Animal Science, 2005; Yokoyama, 1979). Lung volume in rabbits increases with age in contrast to that in humans and dogs which decreases with age.

Bronchial-associated lymphoid tissue (BALT) is present as a distinct tissue (Suckow et al., 1997; Biology and Medicine of Rabbits and Rodents, 1995). The thymus, which persists in the adult, lies ventral to the heart and extends forward into the thoracic inlet.

In rabbits placed in dorsal recumbency while under anesthesia, the V_A/Q ratio scatter is large, particularly for its small size and body weight. No, or minimal shunt, was found and the rabbit has good collateral ventilation (Handbook of Laboratory Animal Science, 2005).

Ferret

The thoracic cavity of the ferret is cone-shaped, narrows cranially and widens caudally. There are generally 14 ribs to the rib cage and 9 sternebrae with the last several ribs not meeting the sternum. The trachea has approximately 60-70 rings of "C" shaped hyaline cartilages and is about 9.0 cm long and approximately 0.5 cm diameter. The gap at the dorsal borders of the hyaline cartilage is filled by smooth tracheal muscle. The trachea bifurcates into left and right bronchus at the level of the 5^{th} intercostal space. Each bronchus subdivides into lobar or secondary bronchi which are further divided into small lobules of the lung. The lungs are approximately 3 times the predicted size based on body weight compared to other mammals (Biology and Diseases of the Ferret 2^{nd} Ed., 1998).

The left and right lungs of the ferret extend approximately from the apex of the thoracic inlet at the 1^{st} and 2^{nd} intercostal space to the 10^{th} and 11^{th} intercostal space adjacent to the diaphragm. The left lung is composed of the cranial and caudal lobes (extends from 6^{th} and 7^{th} to 10^{th} and 11^{th} intercostal space) separated by an oblique fissure. The right lung is composed of the cranial, middle, caudal, and accessory

lobes. Ferret lungs are similar to human lungs anatomically since the lung structure contains excess submucosal glands in the bronchial wall and extra terminal bronchioles. The higher degree of bronchiolar branching and more extensive bronchial submucosal glands, compared to the dog, suggests the ferret as a species for consideration for pulmonary studies (Laboratory Animal Medicine, 2002, 1984; Biology and Diseases of the Ferret, 1998).

Canine

The dog is commonly used in safety evaluations of drugs intended for human use. Considerable scientific documentation exists for the canine as a predictive animal model for humans and an extensive biochemical and physiological database for beagles and other breeds are available. Dogs exhibit similar cardiovascular anatomy and physiology to that in human beings. As with other species the air enters the naris, (nostrils) continues through the turbinates to the nasal cavity, pharynx, larynx, trachea, multiple generations of bronchi, multiple generations of bronchioles and ultimately ends at the alveoli for gas exchange (Handbook of Laboratory Animal Science, 2005; Laboratory Animal Medicine, 2002, 1998).

Dogs do not have sweat glands, except on the pads of the paws. Because dogs do not sweat through the skin, the respiratory system also plays an important role in regulation of temperature. Rapid breaths, termed panting, exchange the warm air from the body for the cooler outside air. Additionally, moisture within the respiratory system evaporates, further cooling these surfaces. Dogs have a left and right lung which is divided into cranial right and left lobes, middle right and left lobes, and caudal right and left lobes (Handbook of Laboratory Animal Science, 2005; Meyer et al., 1989; Laboratory Animal Medicine, 2002, 1984). During anesthesia, the efficiency of oxygenation while in dorsal recumbency under anesthesia, is decreased slightly compared to humans. The alveolar ventilation and perfusion (V_A/Q) match is better than in humans with narrow unimodel distribution of V_A/Q, a small scatter of V_A/Q ratios, and no shunt (unlike developed in humans) (Handbook of Laboratory Animal Science, 2005).

Swine

The lungs of the pig (domestic and minipig) have seven lobes, composed of right and left apical/cranial, middle and caudal/diaphragmatic lobes with an additional accessory lobe for the right lung.

The interlobular fissures are incomplete in the pig. The larynx is prominent with a large vestibule and lateral and middle ventricles that create a caudal narrowing of the structure. The trachea courses from approximately C3-C4 into the thorax. The apical lobe of the right lung has a bronchus that stems from the trachea cranial to the tracheal bifurcation which supplies the other lobes of the lung. The bronchial tree divisions are typical of other species (Laboratory Animal Medicine, 2002, 1984; Bollen et al., 2000; Swine in the Laboratory: Surgery, Anesthesia, Imaging and Experimental Procedures, 2007; The Minipig in Biomedical Research, 2012; Surgery, Anesthesia and Experimental Techniques in Swine, 1998; Swindle et al., 1986).

Functional studies of the airway, including neurochemical anatomy and smooth muscle function, make them useful in models of acute respiratory distress syndrome and asthma (Martin, 1994). Studies of pulmonary tissue and circulation from fetal life to six months of age in domestic swine have shown morphological parameters and functional similarities to humans. Pig pulmonary function matures by 2 weeks of age, however growth and remodeling continue through adult life. Pulmonary tissues are friable and must be handled gently during thoracic surgery. Over inflation of the lungs with a respirator can cause alveolar rupture and emphysematous bullae. These air bubbles can extend into the abdominal mesenteric tissues and result in pneumotosis intestinalis. Tidal volume is approximately 10-15 mL/kg and inflation pressure on a respiratory unit should not exceed 18-20 cm H_2O. The respiratory rate decreases with age from approximately 40 breaths/min in the neonate to 15 breaths/min in an adult (Swine in the Laboratory: Surgery, Anesthesia, Imaging and Experimental Procedures, 2007; Basic Surgical Exercises Using Swine, 1983).

Metabolically, the swine P450 system is more similar to humans than most species including primates (Swindle, 2007). The cytochrome P450 enzyme activity is similar to humans except for low levels of CYP2C and absences of CYP2D. Functionally the liver is similar to humans with some anatomical differences not presented here. These differences are considered to be of minimal relevance when considering cyanide toxicity (Skaanild et al., 1997; Pennington et al., 1988; Swine in the Laboratory: Surgery, Anesthesia, Imaging and Experimental Procedures, 2007).

The V_A/Q matching in swine under anesthesia has greater scatter of the V_A/Q ratios making it less efficient than a dog. Development of some degree of shunt has been reported. When challenged with methacholine (MCH) for the bronchial provocation tests, the pigs did not produce a low V_A/Q match as in dogs (Kubo et al., 1985). This is probably due to the collateral circulation.

The cardiovascular and pulmonary systems in swine share important anatomical and physiological characteristics with humans, making them useful models. Besides size and morphological characteristics, physiological similarities in coronary blood flow, growth of cardiovascular system, and neonatal pulmonary development are also similar to humans (Swindle, 2007). The cardiovascular system is similar to humans anatomically for the left azygous (hemi-azygous) vein which drains the intercostal system into the coronary sinus. The pig coronary system is similar in anatomy and function to 90% of the human population, and the heart in a 40-50 kg miniature pig is the same size as that of an adult human. The blood supply to the conduction system is from the caudal septal artery and thus is predominantly right side dominant like the human. In addition; the pig's aorta contains a vaso vasorum similar to humans. Healing of the myocardium, including injury due to infarctions, is almost identical to humans (Swine in the Laboratory: Surgery, Anesthesia, Imaging and Experimental Procedures, 2007; Gootman, 2001; Swindle et al., 1986; Gardner et al, 1988; Swine in Cardiovascular Research, 1986).

Slight differences reported with the cardiovascular system of swine compared to humans are: 1) the pig heart is more neuromyogenic in its conduction system because of the sparseness of nerve cells in the fibers, the large number of adrenergic cholinergic fibers in the AV node, and right and left bundle branches; 2) the endocardium and epicardium are activated simultaneously due to the difference in the conduction system (humans are activated from epicardium to endocardium), 3) the Purkinje system has large subendocardial fibers (in humans the Purkinje system is less dense than pigs and barely break the endocardial surface) also contributing to the neuromyogenic conduction of the pig (Gardner et al., 1988; Gootman, 2001; Surgery, Anesthesia and Experimental Techniques in Swine, 1998; Swine in Cardiovascular Research, 1986), 4) the rate of conduction in pigs is faster than humans of similar maturity, 5) there are no preexisting collateral vessels in the myocardium (in humans, there is some collateralization and humans can add collateral vessels) (Zhao et al., 2001), 6) blood vessel and the atria in swine tend to be more friable than humans, and

7) peripheral vessels are generally deeper in the pig than most species, to include the human. Peripheral vessels in pigs have valves similar to humans which may cause problems with passage of catheters into small peripheral vessels of the extremities (Swine in the Laboratory: Surgery, Anesthesia, Imaging and Experimental Procedures, 2007). Pig lungs contain a large number of pulmonary intravascular macrophages (PIMS) as opposed to rodents, dogs, primates, and humans. The PIMS remove circulating particles, cell debris, bacteria, or endotoxin from the blood, once activated by pathogen phagocytosis (Brain et al, 1970). PIMS can also produce inflammation in the lung microvasculature, making pigs a good model for acute respiratory distress syndrome (ARDS).

Nonhuman Primate

Inhalation studies have been conducted on non-human primates for multiple disease (bacterial and virus initiated), toxic substances (asbestos, smoke, particulates, etc.) and, toxins (ricin). A key point is the similar anatomy and upright positioning to humans. As with other mammalian species, the general organization of the respiratory system is the same, however, anatomical and cellular differences may result in differing responses to a given stimulus or biological incident in the non-human primate. The Macaque species has been one of the main models used in studies of the respiratory system or for drug testing and will be the main focus of this section.

Anatomically, air flows into the nares, through the nasal vestibule (surrounded by cartilage and lined with squamous epithelium) through the narrow nasal valve (ostium interim) into the nasal chamber divided by nasal septum and into the nasopharynx. Each nasal passage way in the main chamber is defined by a lateral wall, a septal wall, roof, and floor. The lumen of the main chamber is lined with a well vascularized and innervated mucous membrane covered by continuous layer of mucous. Ciliated cells move the mucous toward the oropharynx, and where it is swallowed. Nasal turbinates (increases surface area) consist of bony structures from the lateral wall and lined with well vascularized mucous tissue, which project into the main chamber. Only one pair of sinuses, the maxillary sinus, are present in the macaque monkeys.

The composition of the surface nasal epithelium of the rhesus differs from the other species by the distribution in epithelial populations and types of nasal epithelium. These are squamous epithelium (nasal vestibule), ciliated respiratory epithelium (main chamber and nasopharynx), nonciliated transitional epithelium (between squamous and respiratory epithelium), and olfactory epithelium (dorsal or dorsocaudal aspect of nasal cavity). Olfactory epithelium consists of sensory, sustentacular and basal cells. The olfactory sensory cells (receptor cells) are of bipolar neurons interposed between two sustentacular cells. The dendritic portions extend above the epithelial surface and terminate into a bulbous olfactory knob with protruding immotile cilia of 12 or more. These cilia provide an extensive surface area by enmeshing with each other and the microvilli in surface fluid for reception of surface odorants. The axons of the olfactory sensory cells originate at the base of the cell and pass through the basal lamina, joining axons from other sensory cells to form nonmyelinated nerves in the lamina propria. The axons perforate the cribiform plate to synapse with the neurons in the olfactory bulb of the brain. The olfactory sensory cell has the ability to regenerate from the progenitor cell or stem cells believed to be the basal cells. Sustentacular cells support the sensory cells and are abundant with endoplasmic reticulum (SER) and xenobiotic metabolizing enzymes (such as esterases, cytochrome P-450). This metabolizing function may be instrumental at detoxifying inhaled xenobiotics and function of smell. Bowman's glands are structures within the lamina propria and interspersed among the olfactory nerve bundles comprised of small compact acini in a simple tubular–type gland. The ducts from these glands extend through the olfactory epithelium to the luminal surface after traversing the basal lamina at regular intervals. Copious amounts of neutral and acidic mucosubstances from Bowman's gland contribute to the mucous layer of the luminal surface (Plopper et al., 2005). The metabolizing capability of the olfactory epithelium was reported as being greater than the respiratory epithelium for most animal species Dahl et al., 1991. Sustentacular cells in the olfactory epithelium and Bowman's glands in the lamina propria tend to have high concentration of metabolizing enzymes as evidenced by immunohistochemistry. Squamous epithelium lines the nasal vestibule, which is stratified epithelium composed of basal cells along the basal lamina and several layers of squamous cells that progressively flatten toward the luminal surface. Most likely functions as protective layer similar to skin Nasal transitional epithelium is a narrow strip of nonciliated, microvilli-covered surface epithelium, located

distal to the stratified squamous epithelium and proximal to the ciliated respiratory epithelium. Distinctive features of this cell type in humans and animals are: 1) anatomical location in proximal aspect of nasal cavity between squamous and respiratory epithelium, 2) nonciliated cuboidal or columnar surface cells and basal cells are present, 3) scarcity of goblet mucous cells and paucity of intraepithelial mucosubstances, and 4) an abrupt border (morphological) with the squamous epithelium and less pronounced border with the respiratory epithelium. . The transitional epithelium in monkeys is 4-6 cell layers thick (considered thick), stratified, and composed of 5 different cell types. Respiratory epithelium (ciliated respiratory cells) lines approximately 75 percent of the rhesus nasal cavity. This pseudostratified nasal epithelium is similar to the ciliated respiratory epithelium, but does have several distinctive features Respiratory epithelium lines approximately 75 percent of the nasal cavity (Plopper et al., 2005).

Bonnet monkeys have similar respiratory epithelium to that of other mammals with the composition being primarily ciliated cells, mucous goblet cells, and basal cells. The respiratory nasal epithelium of macaques also contains mucous granule cells and cells with intracytoplasmic lumina, but no brush cells, as present in the rat. The Vomeronasal organ (Jacobson's organ) is highly variable in the primate species. It is well developed in New World monkeys and prosimians and is covered with sensory epithelium. The vomeronasal organs identified in chimpanzees and humans are bilateral septal tubes lined only with nonsensory ciliated epithelium, hence nonchemosensory homologues. Macaques do not have structures resembling vomeronasal organs. Immune tissues, such as lymphocytes, plasma cells (production of antibodies against inhaled antigens or infectious agents) and mast cells, are scattered throughout the lamina propria of nasal mucosa. NALT is more prevalent in monkeys than rodents and is located on the lateral and septal walls of the proximal nasopharynx in primates. The lymphoepithelium, consisting of lymphoid cells and nonciliated cuboidal cells with luminal microvilli covers the luminal side of NALT. Few to no mucous cells or ciliated cells are in this specialized airway. Nonciliated cuboidal cells may assist in the uptake and translocation of inhaled antigen from the nasal lumen to underlying lymphoid structures. Inhaled air and nasal secretions pass over the NALT strategically placed at the entrance of the nasopharyngeal duct, presumably for regional immune defense (Plopper et al., 2005; Smith, et al., 2001; Harkema et al., 1987b).

The nasal and oral pathways are connected to the laryngeal airway by the pharynx. The pharynx in humans and nonhuman primates (rhesus) lies caudal to the nasal cavity, mouth and larynx. The pharynx is anatomically divided into the nasal, oral and laryngeal regions. The nasopharynx is lined with ciliated epithelium with mucous goblet cells. The oropharynx and laryngopharynx are lined with non-keratinized squamous epithelium (Plopper et al., 2005).

The larynx of a rhesus macaque is organized like most mammals in that they have five primary cartilages: 1) the epiglottis, 2) the u-shaped thyroid cartilage, 3 and 4) paired arytenoid cartilages with muscular, vocal and corneal processes, and 5) the ring-shaped cricoid cartilage. In the rhesus and humans, the cuneiform cartilage is in the aryepiglottic fold interspersed between arytenoids and epiglottic cartilages. Principle paired muscles are the lateral and posterior cricoarytenoideus, cricothyroideus, and vocalus along with the unpaired arytenoideus transversus. The epiglottis, false vocal cords and arytenoid cartilages are lined with stratified squamous epithelium. Distal to the free margin of the false vocal cords begins the respiratory epithelium with mucous goblet cells, basal cells, and ciliated cells. The boundary between stratified squamous epithelium and respiratory cells varies with age. Laryngeal sacs found in rhesus (not in humans) are located in the hyoid apparatus and opens through an ostium into the lateral aspect of the anterior larynx (Hilloowala et al., 1978, Sutton et al, 1977; Stearns et al., 1982).

The lung is further divided into tracheobronchial airway and gas exchange airway. The tracheobronchial airway consists of the trachea and two main bronchi (to supply each lung) separating to supply air to the lobar bronchi supplying air within each lung with further branching (generations) throughout each lung until the alveoli are reached for gaseous exchange. The number of generations of the branching of the bronchial pathway varies between species (Bal et al., 1988; Plopper et al., Hill, 1960; Swindler et al., 1973). The lung volume of the rhesus monkey is about 8 percent of its body weight (Ménache et al., 1997).

The trachea enters the thoracic cavity and divides into two primary bronchi in the mediastinum. In adult rhesus monkeys, the trachea is approximately 0.9 cm wide, 9.5-11 cm long, consists of approximately 27 C-shaped cartilaginous tracheal rings, and flattened transversely. The dorsal portion of the trachea is incomplete and is formed by a fibroelastic membrane. Also, the space between the cartilaginous rings

consists of a fibroelastic membrane termed annular tracheal ligament. The ends of the incomplete tracheal rings are joined by smooth muscle, the trachealis muscle. The right bronchus extends almost straight, while the left bronchus extends at a distinct angle (Plopper et al., 2005; Silverman et al., 1980).

The rhesus lung most closely resembles that of the dog with thicker pleura than rat and mouse, and thinner than humans; minimal interlobular separation; and similar arrangement of vessels. In the rhesus macaque, the pulmonary lobation is similar to the dog and cat. The left lung is divided into cranial and caudal lobes with the cranial lobe subdivided into cranial and caudal segments. The right lobe of the lung is divided into four lobes: cranial, middle, caudal, and accessory. Macaque lung bronchi are developed to at least three generations before the first bronchiole is reached. Bronchi open into terminal bronchioles approximately 2-3 mm in length and at least developed to one generation lined with pseudostratified columnar epithelium. Intermediate bronchioles are generally not observed in the rhesus. Other macaques have terminal bronchioles developed to 3-4 generations and lined with simple cuboidal epithelium. The pulmonary parenchyma of macaques has an accentuation of the interstitial densities resulting in diminution of the distinctive bronchovascular markings. Care must be taken to not overemphasize the interstitial pattern and diagnose interstitial pulmonary disease since the radiograph may be normal or secondary to pulmonary ascrids. The tracheobronchial airway, conducting airways from the trachea and two main bronchi to the gaseous exchange area, are comprised of multi-generation branching tubes and represent approximately 1.8 percent of the lung volume. The bronchi which are the proximal portion of these branching tubes are characterized by their histological composition. Histologically, there is a mucous presence and basal cells in the epithelium, mucosal glands in the interstitium, and a significant amount to cartilage in the interstitial spaces. The distally the airway branches are termed bronchioles, where the walls are thinner, reduced complexity of the airway epithelial population, little to no cartilage in the smooth muscle wall of the bronchiole. The airway pattern and organization varies between species in that for the rhesus the airway branches in the proximal aspect of the tracheobronchial tree come off at a greater angle than in rodents but the distribution pattern is similar. Cartilage in the tracheobronchial airways in the rhesus is found in the walls from the trachea distally to the smallest bronchioles. Cellular composition in the primate shows, as in all species, the C-shaped cartilages and band of smooth muscle joining the open ends is in the interstitium

of the tracheal wall. The trachea and proximal airways have extensive sub-mucosal glands beneath the epithelium in primates and humans. The alveolar gas exchange region comprises the majority of the lung. Greater than 90 percent of the primate lung volume is alveolar gas exchange area with approximately 80 percent of this volume being alveolar air space. The size of the alveoli varies in proportion to the body size, being larger for larger species. Approximately 10 percent of all tissue in the lung is comprised of the cellular and acellular portions of the tissue surrounding the alveolar air pockets. The remaining tissue is comprised of blood vessel walls and conducting airways. Alveolar walls on the air side consist of capillary epithelium, connective tissue and alveolar epithelium. The blood air barrier cellular and acellular compartment is thicker in primates than that of rodents. The alveolar blood-air barrier epithelium constitutes about on-quarter of this thickness in primates and nearly one-third in rodents. Capillary endothelial cells make-up about 25 percent of the blood-air thickness and this is much less in humans. A larger proportion of the barrier is made up by the acellular interstitial compartment in larger species with a range of 40 percent in mice to 60 percent in humans (Wolfe-Coote, 2005; Valverde et al., 2005; Plopper et al., 2005; Silverman et al., 1980).

Comparative Respiratory Systems

The respiratory system and thoracic cavity of animals and humans differ in anatomy and function. In general, Monkeys and humans have generally simple noses with breathing as the primary function. More complex noses are found in dogs, cats, and rodents where olfaction is a primary function. Humans and primates can breathe through the mouth and nose. Rodents, such as mice, rats, hamsters, and guinea pigs, are obligate nose breathers because the epiglottis is in close apposition to the soft palate (Wolfe-Coote, 2005).The human has three turbinates (superior, middle, and inferior), while the rhesus has a dorsal ethmoturbinate and a ventral maxilloturbinate. Other mammals (dog, cat, rabbit, and rodents) have more complex folds and branching patterns in their turbinates. The complex maxilloturbinates of small laboratory rodents and rabbits provide protection for the lower respiratory tract through an extensive surface area for filtration, absorption, and disposal of particles and gases. This ability is not present with the simple turbinate scrolls in the human and rhesus monkey. Another difference between species is observed in distribution of

nasal epithelium populations and the cell types that make-up these populations. These cell types consists of 1) squamous epithelium (mainly restricted to the nasal vestibule), 2) ciliated respiratory epithelium (main chamber and nasopharynx), 3) nonciliated transitional epithelium (lying between squamous and respiratory epithelium in the proximal or anterior aspect of the main chamber), and olfactory epithelium (in the dorsal or dorsocaudal aspect of the nasal cavity). For example, the percentage of nasal airway covered by olfactory epithelium varies between animal species. In rodents and rabbits and some of the other small animal laboratory species, the olfactory function is key as evident from the design of the complex shape of the ethmoturbinates and the cellular lining, predominantly olfactory neuroepithelium with the highest concentration in the distal half of the nasal cavity. In rats, approximately 50 percent of the nasal cavity surface area is the neurogenic sensory epithelium and in adult rhesus, about 14 percent is covered, while in humans only 3% (500 mm^2) is covered. Mice, rabbits and dogs are closer to rats than humans with respect to the relative amount of nasal epithelium in their nasal cavity (Plopper et al., 2005; Gross et al., 1982; Woolf-Coote, 2005; Sorokin, 1988).

Discrete larger focal sites of lymphoid tissue termed nasal associated lymphoid tissue (NALT) are located in the nasopharyngeal mucosa of humans, laboratory animals and primates and are important in the regional immune defense of the upper airways (Plopper et al., 2005). In humans, the correlate to NALT is Waldeyer's ring, the oropharyngeal ring of lymphoid tissue composed of the adenoid and bilateral tubular palatine and lingual tonsils (Brandtzaeg, 1984). The pharynx in humans and nonhuman primates (rhesus) lies caudal to the nasal cavity, oral cavity and larynx. In other laboratory species (rodent and dog), the anterior portion of the nasopharynx lies ventral to the caudal aspect of the main nasal cavity, the pharynx is distal to most of the nasal airway and dorsal to the oral cavity and larynx. The pharynx is a musculomembranous tube of approximately 130 mm in an adult human, 115 mm in an adult male beagle dog, 35 mm in an adult male rhesus monkey, and 22 mm in an adult Sprague-Dawley rat (Schreider et al., 1981). Differences between the human larynx and that of the rhesus monkey is the presence of the laryngeal sacs, size of the larynx and internal passage, and position of larynx in cervical region (Hilloowala et al., 1978; Fitch, 1997; Petra et al, 1986; Schreider et al, 1981; and Flugel et al., 1991).

The lung volume comparison between the monkey, mouse, rat and humans are estimated as 0.082, 0.048, 0.032, and 0.059 respectively (lung volume/body weight). The lung volume reported for the same species based on fixed tissue in mL is as follows: 2,393 ± 100 mL for monkey, 1.1 ± 0.05 mL for mouse, 11.4 ± 1.2 mL for rat, and 4,341 ±285 mL for human. The rhesus monkey has six lung lobes whereas the rat, mouse, and human have 5 lobes. The lungs are lined with a connective tissue band of mesothelial surface facing the pleural space. The pleura is thin for the monkey, mouse and rat, but thick in the human. Intralobular connective tissue is minimal for the monkey, minimal, if any for the rat and mouse but extensive for the human. Other species such as pigs, cows and horses also have extensive interlobular and intralobular connective tissue that joins major vessels and bronchi to the plural surface. Rhesus have little interlobular connective tissue. In general, the comparative thickness of the pleura tends to be thin in the rhesus monkey, but thicker than rats and mice and thinner than humans. Cartilage is restricted to a small zone in the bifurcation area of distal bronchioles of rhesus and humans, whereas, in the rodent species the cartilage ends in the lobar region. The intrapulmonary airways in all species have multiple generations before gas exchange with a significant number being thin walled and with minimal cartilage (e.g. non-respiratory bronchioles). In carnivores and primates the transition zone between conducting airways and gas exchange separate their lungs from other species in that the distal airway walls have a mixture of bronchiolar epithelial sub-populations and populations mixed with alveolar gas exchange area. The conducting airway branching ranges between 13-17 generations for the monkey and 17-21 generations in the human with most of the other species being the same as the monkey. In primates the branching of the airways tends to come off the parent airway at a 45 degree angle and uniform and equal in size and diameter which is termed dichotomous branching. In humans and monkey, the branching pattern of the tracheobronchial airways is dichotomous (also trichotomous for some primate species) and that of rodents is monopodial. The cellular composition varies between species from the trachea to the alveolar gas exchange. All species have the C-shaped cartilage and band of smooth muscle joining the ends of the cartilage over the open area in the interstitium of the wall of the trachea. For rhesus monkeys and humans, the trachea and proximal airways have extensive sub-mucosal glands beneath the epithelium. The presence of these glands is variable in the smaller laboratory animal species. Species differences are observed in the

substantial amount of epithelium that lines the tracheal luminal surfaces, the composition of the epithelium, the density of cells lining the surface, and proportion of cell phenotypes in the epithelium. The thickness of the tracheal epithelium in rhesus is about twice that of the rat and mouse and half to one-third of humans. In primates, a large percentage of the airway consists of mucous cells and in mice and rats these cells are generally not found in the trachea of healthy animals. The proportion of ciliated epithelial cells is similar among all species, however, variation in basal cell proportion among species is observed. With differences in cellular populations, the composition of secretions and thus carbohydrate content is also variable among species. Primates have heavily sulfated secretory products that are not usually found in mammals. The organization of composition of the proximal airways to the distal airways varies greatly between the mammalian species. Smooth muscle is in the airway walls of all mammalian species, but as you progress distally the amount of cartilage and submucosal glands varies significantly. The lobar airway region in the rodent species is absent and both are present in the monkey and humans. Primates have a predominance of mucus and basal cells in their epithelial populations, whereas in rodents Clara cells are the prominent nonciliated cell population. These differences become more marked as you progress distally. The bronchioles of the distal conducting airways have as the main difference between species is related to the epithelial surface lining. Clara cells are the primary secretory cell phenotype, no mucous cells and basal cell numbers in the epithelium is related to the degree of alveolarization for the rodent species. In primates (rhesus monkey), the bronchioles have extensive smooth muscle portions arranged in bundles interspersed with connective tissue, which is not seen in other laboratory mammals. The alveolar gas exchange region comprises the majority of the lung. Greater than 90 percent of the primate lung volume is alveolar gas exchange area with approximately 80 percent of this volume being alveolar air space. The size of the alveoli varies in proportion to the body size, being larger for larger species. Alveolar surface area is related to metabolic body weight. The capillary bed, which lines the individual alveoli in the septa, has a surface area comparable to the alveolar surface area for adequate transport of gas to and from the exchange surface. Capillary blood volume varies in relation to the extent of gas exchange area (Plopper et al., 2005).

Conclusions

The amount of cyanide, duration of exposure, and premorbid condition of the individual influence the time to onset and severity of illness. A critical combination of these factors overwhelms endogenous detoxification pathways, allowing cyanide to diffusely affect cellular function within the body. No reliable pathognomonic symptom or toxic syndrome is associated with acute cyanide poisoning. Clinical manifestations reflect dysfunction of oxygen-sensitive organs, with central nervous and cardiovascular findings predominating. For HCN gas, the time to onset of symptoms is typically in seconds resulting in a narrow window for therapeutic intervention.

The size, shape, and specie of the animal affects the pulmonary system's anatomy, physiology and function of the pulmonary system. For instance; respiratory functions of larger animals are less efficient in dorsal recumbency versus sternal recumbency, affecting the respiratory (breathing) pattern, alveolar ventilation and perfusion of alveolus, which affects dosimetry of CN as well as anesthesia, if used. Differences in epithelium lining composition, proportion of respiratory tract covered by a specific cell population, and extent of the metabolic activity of the cell types within the pulmonary system affects CN metabolism, particularly detoxification, which may affect interpretation of results. The degree to which the pulmonary system metabolizes inhaled CN in comparison to the liver is unclear and needs further study.

CN intoxication is complex as it inhibits multiple enzyme systems, affects other biochemical systems and physiological functions. While there are numerous acceptable models of intoxication, a number of factors limit the comparability of some of these models to the clinical scenario, particularly when evaluating the efficacy of an antidote. One of the most important factors is allometry (Ballantyne et al., 2008; WHO, 2004). Generally speaking, the smaller the animal, the more rapid the metabolism of xenobiotics, leaving the "window" between onset of severe intoxication and therapeutic intervention to either recovery or death quite narrow in some cases. While this issue has not been specifically referred to in the published literature with regard to cyanide, it theoretically limits the capacity for effective administration of antidotes after intoxication, which corresponds to the clinical situation. Because of this, many researchers have pre-treated animals with antidotes, a situation which has few clinical correlates and

has been justly criticized (Ballantyne, 1987a). Additionally, volume administration, (antidote), excipient effects (solvents for the toxicant and antidotes), and volume depletion (blood sampling), are of relatively greater importance when dealing with very small animals. Blood sampling requirements for analytical testing are such that definite limitations exist for smaller species.

In general, the primary mechanism of acute inhalational CN toxicity is the result of inhibition of cytochrome c oxidase, the terminal oxidase of the respiratory system, resulting in cytotoxic hypoxia. The intracellular hypoxia resulting from CN^- complexing with the ferric iron of the mitochondrial cytochrome c oxidase, inhibits the electron transport chain and oxidative phosphorylation, resulting in anaerobic metabolism with a decrease in ATP production and increase in lactic acid production (Casarett and Doull's Toxicology: The Basic Science of Poisons, 3rd Edition, 1986; WHO, 2004). The brain and myocardium are the tissues with the greatest oxygen demand and thus, most sensitive, and most rapidly and markedly affected. The CN binds with both the reduced and oxidized forms of cytochrome a_3 component of cytochrome c oxidase (Raza et al., 1994). The stable oxidized form of the oxidized enzyme-CN complex can become reactivated when in the presence of reducing equivalents and CN dissociates from the enzyme-inhibitor complex reactivating the enzyme (WHO, 2004). This reversible nature of the inhibition of the enzyme is the basis for the use of certain antidotes, which shift the equilibrium of CN from intracellular to the plasma compartment. These antidotal effects are observed with cyanomethemoglobin (CNMetHb) formation, and chelation of CN or conversion to thiocyanate (SCN). CN toxicity is mediated by intramitochondrial mechanisms causing cytotoxic hypoxia and since erythrocytes sequester CN, the plasma CN concentration is the primary determinant of cytotoxicity (Beasley et al., 1988). The main pathophysiological cause of CN-induced lethality is inhibition of the central regulatory mechanisms of respiration and cardiotoxicity (WHO, 2004).

Rodents for the small animal species selection may be used for general toxicity and proof of concept studies. As a inhalation cyanide model, there is a large historical reference data base, the availability of a wide range of animals that are genetically diverse and well characterized, ready availability, and low cost compared to most animals.

For the larger species, the dog, mini-pig, pig and non-human primates are most commonly used. The model used will depend on the endpoints to be evaluated, the ability of utilized larger species, and the anatomical, physiological and metabolic similarities to the human.

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